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Plasmodium falciparum erythrocyte membrane protein 1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule 1

(malaria/variant antigen)

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ABSTRACT **Adherence of mature *Plasmodium falciparum* parasitized erythrocytes (PRBCs) to microvascular endothelium contributes directly to acute malaria pathology. We affinity purified molecules from detergent extracts of surface-radioiodinated PRBCs using several endothelial cell receptors known to support PRBC adherence, including CD36, thrombospondin (TSP), and intercellular adhesion molecule 1 (ICAM-1). All three host receptors affinity purified *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a very large malarial protein expressed on the surface of adherent PRBCs. Binding of PfEMP1 to particular host cell receptors correlated with the binding phenotype of the PRBCs from which PfEMP1 was extracted. Preadsorption of PRBC extracts with anti-PfEMP1 antibodies, CD36, or TSP markedly reduced PfEMP1 binding to CD36 or TSP. Mild trypsinization of intact PRBCs of *P. falciparum* strains shown to express antigenically different PfEMP1 released different ^{125}I-labeled tryptic fragments of PfEMP1 that bound specifically to CD36 and TSP. In clone C5 and strain MC, these activities resided on different tryptic fragments, but a single tryptic fragment from clone ItG-ICAM bound to both CD36 and TSP. Hence, the CD36- and TSP-binding domains are distinct entities located on a single PfEMP1 molecule. PfEMP1, the malarial variant antigen on infected erythrocytes, is therefore a receptor for CD36, TSP, and ICAM-1. A therapeutic approach to block or reverse adherence of PRBCs to host cell receptors can now be pursued with the identification of PfEMP1 as a malarial receptor for PRBC adherence to host proteins.**

Mature stages of *Plasmodium falciparum* parasitized erythrocytes (PRBCs) sequester from the peripheral circulation by adhering to postcapillary venular endothelium within various organs including heart, lung, liver, and brain (1). Adherence of PRBCs to endothelial cells often causes microvascular occlusion (2). Occlusion of cerebral blood flow probably contributes directly to the acute pathology of human cerebral malaria (1–3). PRBC adherence to endothelial cells is mediated by several different endothelial cell surface receptors including CD36, thrombospondin (TSP), and intercellular adhesion molecule 1 (ICAM-1) (3, 4). Vascular cell adhesion molecule 1 (VCAM-1) and endothelial leukocyte adhesion molecule 1 (ELAM-1) can also act as endothelial cell receptors for a minority of *P. falciparum* parasites (5). The identity of the relevant receptor(s) on the PRBC surface responsible for adherence to endothelium is the subject of debate. Several parasite proteins including Ag332 (6), sequestrin (7), and *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) (3, 4, 8), as well as a modified form of the host protein band 3 (9, 10), have been proposed as candidates.

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PfEMP1 is a high molecular mass antigenically variant malarial protein of 200–400 kDa expressed on the PRBC surface (8, 11, 12). PfEMP1 can be radiolabeled by cell surface iodination of intact PRBCs (13, 14). Extraction of PfEMP1 from PRBCs requires SDS for solubilization of the membrane cytoskeleton (13). The molecular properties of PfEMP1 correlate strongly with the adherence properties of intact PRBCs (3). PRBC adherence of different isolates and laboratory strains is correlated with the expression of PfEMP1 on the surface of mature stage PRBCs (13, 14). ^{125}I-labeled PfEMP1 can be identified on the surface of adherent PRBCs but is lacking from the PRBC surface of nonadherent parasites (13, 14). Alterations in the adherence phenotype of PRBCs or in properties of antibody-mediated PRBC agglutination are often associated with emergence of new forms of PfEMP1 (15, 16). Mild trypsinization of intact mature PRBCs leads to the digestion of the extracellular portion of PfEMP1 and concomitantly ablates PRBC cytoadherence (17). Antibody-mediated blockade or reversal of PRBC adherence is correlated with the ability of the reacting sera to agglutinate the corresponding PRBCs and to immunoprecipitate surface-labeled PfEMP1 (14, 17). Each of these properties can be mediated by strain-specific antibodies. Finally, antisera raised against a recombinant protein derived from a PfEMP1 gene of Malayan Camp (MC) strain parasites block adherence of MC strain PRBCs to CD36 (8). Thus, PfEMP1 is a likely candidate for the PRBC adherence receptor.

In this report, we demonstrate directly that PfEMP1 binds to CD36, TSP, and ICAM-1. Tryptic fragments derived from PfEMP1 bind to CD36 and TSP, and distinctly different domains of PfEMP1 act as receptors for CD36 and TSP.

MATERIALS AND METHODS

Parasites. MC strain *P. falciparum* parasites of two phenotypes [$K^+R^+C^+$ (knob, rosetting, and adherence positive; denoted MC K^+) and $K^-C^-R^-$ (knob, rosetting, and adherence negative; denoted MC K^-], clone C5 $K^+C^+R^-$ of FCR_3 strain (denoted C5), and clone ItG2-ICAM K^+C^+ (ref. 18; denoted ItG) were maintained in culture with O^+ human erythrocytes (8). Adherence of mature PRBCs to CD36 or TSP immobilized to plastic, or to Chinese hamster ovary (CHO) cells transfected with CD36 or ICAM-1, was as described (8, 19). PRBCs of all adherence-positive strains bound to CD36 and TSP. PRBCs of clone ItG also bound to ICAM-1-

Abbreviations: ELAM-1, endothelial leukocyte adhesion molecule-1; HIS, human immune serum; ICAM-1, intercellular adhesion molecule-1; MAb, monoclonal antibody; MC, Malayan Camp; PRBC, parasitized erythrocyte; PfEMP1, *P. falciparum* erythrocyte membrane protein 1; TSP, thrombospondin; TX100, Triton X-100; VCAM-1, vascular cell adhesion molecule-1.

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transfected CHO cells. None of the PRBCs bound to VCAM-1 or ELAM-1.

Antibodies. Mouse monoclonal antibody (MAb) 179 (from Affymax Research Institute) recognizes an epitope sequence incorporated into the carboxyl terminus of CD36, VCAM-1, and ELAM-1 each expressed as a phosphoinositol glycan-linked extracellular domain in stable transformed CHO cells (Affymax Research Institute). Mouse MAbs B-C14 (anti-ICAM-1) and TSP-B7 (anti-TSP) were purchased from Bio-Source (Camarillo, CA) and Sigma, respectively. Rabbit sera J1008 (anti-PfEMP3) and 05-75 (which recognizes PfEMP3 and MC PfEMP1) have been described (20). Human immune serum was pooled from five individuals resident in a *P. falciparum* endemic area of Ghana (20). *Aotus* anti-*P. falciparum* sera 779 and 9025, specific for strain MC K^+, and serum 424C, specific for strain FMG (parent strain of clone FCR_3-C5 maintained in *Aotus* monkeys), were from animals repeatedly infected with blood stage parasites and drug cured (17). *Aotus* anti-MC sera 862 was a gift from the Walter Reed Army Institute of Research. Rat serum (rat #1) against the MC-PfEMP1 recombinant protein, rC1-2, was as described (8).

Soluble Receptors. Soluble CD36, VCAM-1, and ELAM-1 extracellular domains were obtained in the form of harvest supernatant (1–5 μg/ml) after phospholipase C treatment of cultured cells as described (8). TSP was purchased from Gibco BRL. ICAM-1 was extracted from 6.5×10^6 CHO-ICAM cells (19) by a 30-min treatment on ice with 1 ml of 1% Triton X-100 (TX100), 25 mM Hepes, RPMI 1640 (pH 7.3) containing a cocktail of protease inhibitors (20). The cell extract was centrifuged at 4°C for 30 min at 15,000 × *g* to remove insoluble material.

Surface Iodination, Trypsinization, Sequential Extraction, and Immunoprecipitation. Mature intact PRBCs were enriched to >90% by the Percoll-sorbitol method (21), iodinated by the lactoperoxidase method, and sequentially extracted with 1% TX100 followed by 2% SDS (20). For trypsinization, iodinated PRBCs, 10% hematocrit, were incubated with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (10 μg/ml) in phosphate-buffered saline for 5–10 min as described (8), and the tryptic supernatant was collected. Immunoprecipitation was performed using 5–7 μl of SDS extract, 10–15 μl of TX100 extract, or 10–25 μl of tryptic supernatant. Samples derived from immunoprecipitation or affinity purification were solubilized in 5% SDS sample buffer, fractionated by SDS/PAGE using 5–10% gradient acrylamide gels, dried, and exposed to Kodak X-Omat XAR-5 film.

Affinity Purification of PfEMP1. Fifty microliters of GammaBind Plus Sepharose (Pharmacia) was precoated for 90 min at 21°C with 50 μg of MAb 179, 45 μg of MAb TSP-B7, or 20 μg of MAb B-C14. The precoated resin was incubated for 3 hr with 0.5 ml of soluble receptor from the harvest supernatant of CD36, VCAM-1, or ELAM-1; 5 or 10 μg of purified TSP; or 1.25 ml of CHO-ICAM extract, washed three times with 5 ml of binding medium (BM; 25 mM Hepes, 1% bovine serum albumin, 0.5% TX100 in RPMI 1640) at pH 6.7 (for CD36, VCAM-1, and ELAM-1), BM at pH 7.3 (for ICAM-1), or BM with 1 mM $CaCl_2$ at pH 7.3 (for TSP). The immobilized receptors were incubated for 16 h at 4°C with 5–10 μl of SDS extract, 10–15 μl of TX100 extract, or 12.5–25 μl of tryptic fragment supernatant, reconstituted in 5 ml of the appropriate BM containing protease inhibitors. The resin was washed twice with 7.5 ml of cold BM, with a 10-min incubation between each wash, and solubilized with 150 μl of 5% SDS sample buffer.

Preadsorption of PfEMP1. Four hundred microliters of GammaBind Plus Sepharose precoated with 100 μl of J1008 or 05-75 sera, 200 μg of MAb 179, 240 μg of MAb TSP-B7, 50 μg of TSP, or CD36 immobilized from 4 ml of harvest supernatant (7.5–10 μg) was used to preadsorb (16 hr, 4°C) 16 μl of SDS extract derived from iodinated MC K^+ PRBCs, reconstituted in 5 ml of BM. Each of the preadsorbed reconstituted extracts was divided to four 1.25-ml portions and affinity purified (5 hr, 21°C) with immobilized CD36 or immobilized TSP or immunoprecipitated with 5 μl of *Aotus* anti-*P. falciparum* 779A serum or rabbit 05-75 serum. For quantification of the binding results, autoradiographs were scanned using a Bio-Rad model GS-670 imaging densitometer and analyzed with the MOLECULAR ANALYST image analysis software package (Bio-Rad).

RESULTS

Binding of PfEMP1 to CD36. Immobilized CD36 incubated with the SDS extract of the MC K^+ strain of *P. falciparum* affinity purified a single high molecular mass iodinated protein (Figs. 1*A* and 2). The mobility of this protein was identical to PfEMP1 immunoprecipitated from the same SDS extract by rabbit serum 05-75 and *Aotus* anti-MC K^+ serum (Fig. 1*A*) and anti MC-PfEMP1 sera raised against a recombinant fragment of the MC PfEMP1 gene (data not shown). This protein was not affinity purified from the detergent extracts of the non-adherent MC K^- PRBCs (Fig. 1*B*), which do not express detectable surface-exposed PfEMP1 (13). In concordance with the binding properties of MC K^+ strain PRBCs, the MC PfEMP1 protein so identified did not bind to the host receptors ELAM-1, VCAM-1, and ICAM-1 (Fig. 1*A*). The affinity-purified protein was isolated only from SDS extracts, not from TX100 extracts, and was not isolated from detergent extracts (SDS and TX100) of PRBCs subjected first to mild trypsinization (Fig. 2), a treatment known to ablate PRBC adherence and cleave PfEMP1 (17). The major soluble ^{125}I-labeled fragments generated by trypsin treatment were of 130, 100, and 80 kDa (Tf130, Tf100, and Tf80) from the K^+ MC strain in one experiment (Fig. 3*A*) and of 125 (Tf125) and 90 kDa (Tf90) in another (Fig. 3*D*). Each of these major tryptic fragments was immunoprecipitated with rat anti-MC-PfEMP1 sera (Fig. 3*D* and D.I.B., unpublished data), with pooled HIS, or with homologous (Fig. 3*A*) but not heterologous *Aotus* strain-specific sera (data not shown). Hence, these tryptic fragments were derived from PfEMP1 and bear strain-specific epitopes. Tf80 and several smaller tryptic fragments, the smallest about 20 kDa, bound to CD36 but not to TSP or other host receptors (Fig. 3*A*). None of the smaller tryptic fragments that bound to CD36 were immunoprecipitated by strain-specific *Aotus* serum or pooled HIS (Fig. 3*A*). Tf90 bound to CD36 but not to TSP (Fig. 3*D*). The rat anti-PfEMP1 serum that immunoprecipitated Tf90 was raised against the rC1-2 region of the MC strain PfEMP1 gene (8). Binding of this fragment to CD36 but not to TSP is in agreement with the capacity of the rat anti-PfEMP1 serum to block adherence to CD36 but not to TSP. These results also show that the Tf90 tryptic fragment of PfEMP1 contains the rC1-2 region of PfEMP1 (as defined by antibodies) and that it binds specifically to CD36.

Three proteins were affinity purified from parasite clone C5 with CD36 but not with ELAM-1, VCAM-1, or ICAM-1 (Fig. 1*C*). Antigens of the same mobility were immunoprecipitated by pooled HIS and *Aotus* serum specific for strain FMG/FCR_3, the parent strain of clone C5 (Fig. 1*C*). Multiple PfEMP1 bands from clone C5 were also detected by immunoblotting with sera raised against the rC1-2 recombinant fragment of MC strain PfEMP1 (8). The PfEMP1 bands of clone C5 had different mobilities from those of the MC PfEMP1 (Fig. 1*C*). Binding of the ItG PfEMP1 to CD36 was weak but consistently positive compared to negative controls (Fig. 1*D*). A single 105-kDa fragment (Tf105) from clone C5 specifically bound to CD36 (Fig. 3*C*). The larger tryptic fragment of clone ItG-ICAM, Tf150, bound to CD36 and TSP but not to ICAM-1 (Fig. 3*B*).

Several other iodinated molecules, primarily band 3, were purified with PfEMP1 from extracts of clones C5 and ItG incubated with immobilized CD36 (Fig. 1 *C* and *D*) but were absent with strain MC (Fig. 1 *A* and *B* and Fig. 2). Significant amounts of band 3 were also found in immunoprecipitations performed with C5 and ItG extracts (Fig. 1 *C* and *D*). However,

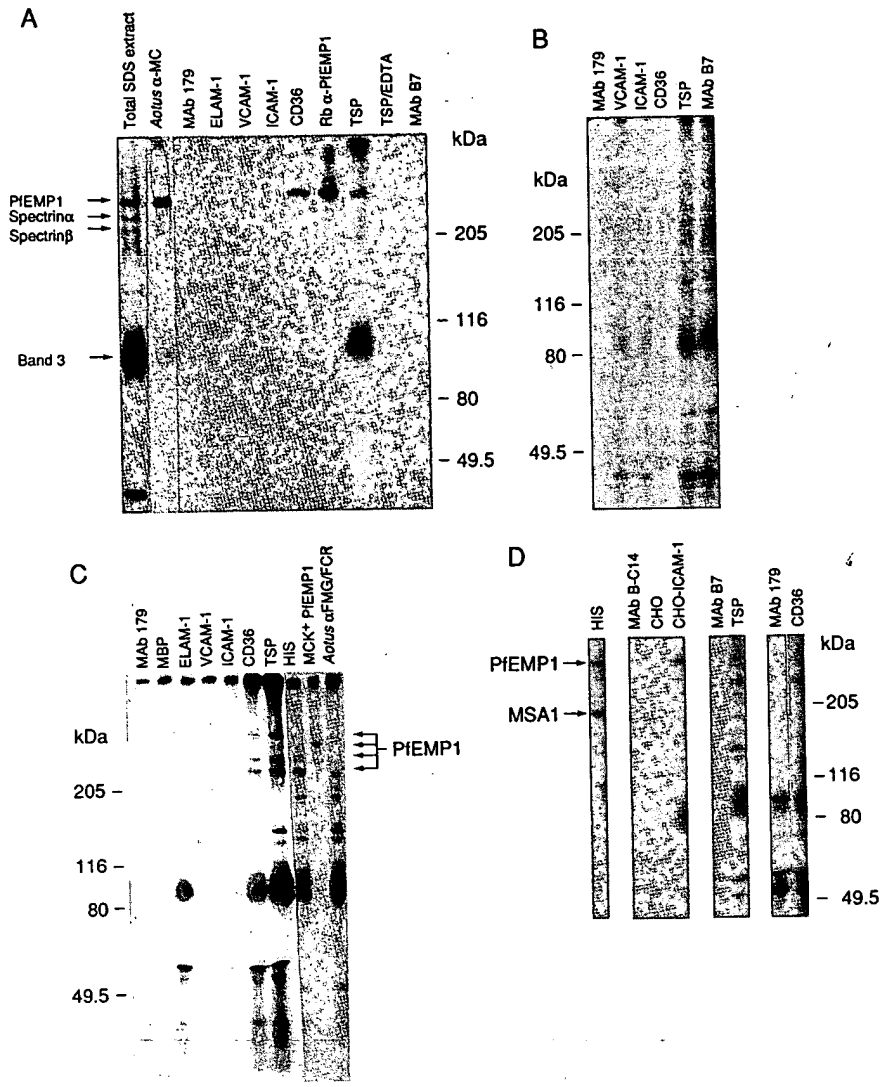

FIG. 1. Autoradiograph of antigens immunoprecipitated or affinity purified with immobilized host cell receptors from total SDS extracts of surface-iodinated PRBCs. (*A*) Strain MC K^+C^+ (adherence positive for CD36 and TSP). (*B*) Strain MC K^-C^- (knobless and adherence negative). (*C*) Clone FCR_3-C5 (K^+, adherence positive for CD36 and TSP). (*D*) Clone ItG (K^+, adherence positive for CD36, TSP, and ICAM-1). Soluble receptors (CD36, VCAM-1, and ELAM-1) and TSP were immobilized on beads via MAb 179 and MAb B7, respectively. ICAM-1 was extracted with TX100 from CHO-ICAM-1 cells and immobilized on beads via MAb B-C14. MSA1, merozoite surface antigen 1 identified by immunoprecipitation with MAbs to MSA-1. HIS, pooled HIS. *Aotus* α-MC and *Aotus* αFMG/FCR are *Aotus* serum 9025 and 424C, respectively. α, anti; Rb, rabbit; MBP, maltose binding protein.

band 3 was not affinity purified from the tryptic supernatants (Fig. 3). It is conceivable that after detergent extraction band 3 can bind to PfEMP1 of some but not all strains (Fig. 1). The PRBC sequential extraction procedure may contribute to this interaction (Fig. 3 vs. Fig. 1). Nonetheless, such interaction does not seem to be involved in binding of PfEMP1 to CD36.

Binding of PfEMP1 to TSP. PfEMP1 from the SDS extracts of strains MC K^+, ItG, and clone C5 bound to immobilized TSP (Fig. 1). No binding was detected from SDS extracts of the nonadherent MC K^- strain (Fig. 1*B*). The interaction of PfEMP1 with TSP required calcium (Fig. 1*A*), was optimal between pH 7.3 and 7.5 (data not shown), and was sensitive to mild trypsinization (Fig. 2), properties shared with those of PRBC adherence to TSP (22). The tryptic fragments Tf130, Tf100 (Fig. 3*A*), and Tf125 (Fig. 3*D*) from strain MC specifically bound to TSP but not to CD36. Tf150 from strain ItG bound to both TSP and CD36 (Fig. 3*C*). The C5-binding domain for TSP appears to have been destroyed by trypsin treatment (Fig. 3*B*). Alternatively, it may reside on unlabeled tryptic fragments.

Radioiodinated molecules other than PfEMP1 bound to TSP (Figs. 1 and 2). However, when tryptic supernatants were examined, only PfEMP1 fragments were detected bound to TSP (Fig. 3). Note that PfEMP1 fragments can be identified by their strain-specific immunoprecipitation by appropriate *Aotus* sera, in parallel with immunoprecipitation of the intact protein. Binding of band 3 to TSP was apparent only in the presence of calcium (Fig. 1*A*). In contrast to binding of PfEMP1, band 3 bound to TSP from extracts derived from adherent and nonadherent (K^- and trypsinized) PRBCs (Figs. 1 and 2). Thus, binding of band 3 to TSP does not correlate with PRBC adherence.

PfEMP1 Binding to ICAM-1. PfEMP1 derived from ItG-ICAM clone *P. falciparum* was affinity purified with immobilized ICAM-1 extracted from CHO-ICAM cells but not with immobilized extracts of CHO cells or by the immobilizing MAb alone (Fig. 1*D*). In contrast, MC PfEMP1 and C5 PfEMP1 did not bind to immobilized ICAM-1, consistent with the fact that MC K^+ and C5 PRBCs do not adhere to ICAM-1.

Expression of Variant Forms of MC-PfEMP1. Variant forms of MC-PfEMP1 are often evident in different experi-

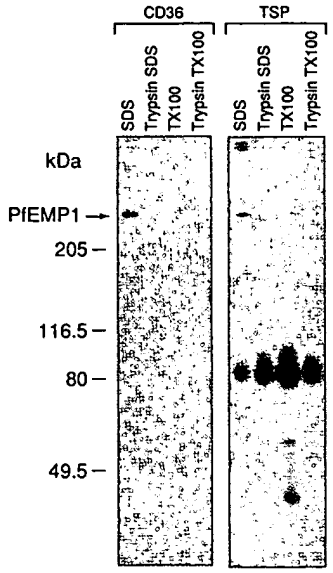

FIG. 2. Autoradiograph of PfEMP1 affinity purified from different extracts of surface-iodinated PRBCs of strain MC K^+C^+ with immobilized CD36 or immobilized TSP. SDS, SDS extract; Trypsin SDS, SDS extract derived from intact trypsinized PRBCs; TX100, TX100 extract; Trypsin TX100, TX100 extract derived from intact trypsinized PRBCs.

ments (Figs. 1–4). Each experiment shown here represents extracts of PRBCs derived from different thaws from a stock of noncloned MC parasites. The MC ^{125}I-PfEMP1 of these extracts displayed a different number of PfEMP1 forms, differences in the SDS/PAGE mobility of PfEMP1 relative to PfEMP2 (data not shown), variability in the size of the tryptic fragments (Fig. 3), and altered sensitivity of PfEMP1 to trypsin (Fig. 2 and D.I.B., unpublished observations). Identification by cDNA cloning and sequencing of MCvar-1 and MCvar-2 PfEMP1 molecules in MC parasites demonstrated simultaneous expression of multiple forms of PfEMP1 (8). Repeated thaws of cryopreserved wild isolates exhibited different agglutination patterns with human sera (23). Thus, with a mixed parasite population, cryopreservation and growth conditions appear to affect the expression of PfEMP1. In Fig. 2, we observed a trypsin-resistant band with slightly lower mobility than the strongly radiolabeled trypsin-sensitive PfEMP1 band. Recently, a trypsin-resistant form of PfEMP1 was described (24), which is involved with adherence of trypsinized PRBCs to N-linked oligosaccharide on the surface of melanoma cells but not to CD36 and ICAM-1. We note that the minor ^{125}I-labeled trypsin-resistant band of the MC strain, approximate size of 300 kDa, bound to TSP but not to CD36 (Fig. 2).

PfEMP1 Is Affinity Purified Specifically by Antibodies, CD36, and TSP. SDS extract derived from strain MC K^+ was preadsorbed using immobilized rabbit anti-PfEMP3 serum (J1008), rabbit anti-PfEMP1 (and PfEMP3) serum (05-75), CD36, or TSP. The preadsorbed SDS extracts were then subjected to affinity purification using antisera or immobilized endothelial cell receptors (Fig. 4). The extent to which PfEMP1 was purified was quantitated by scanning densitometry of the autoradiograms. The intensity of the immunoprecipitated PfEMP1 bands was twice that of PfEMP1 affinity purified with CD36 or TSP (Table 1). Preadsorption with the rabbit anti-PfEMP1 serum reduced the immunoprecipitation of PfEMP1 with the same rabbit serum by 98.4% and by more than 92% with the *Aotus* anti-MC serum. In the precleared SDS extracts, binding of PfEMP1 to CD36 was abolished, whereas binding to TSP was reduced by 73% (Fig. 4 and Table 1). The 27% of the PfEMP1 that was not precleared by rabbit 05-75 serum antibodies and that bound to TSP was associated

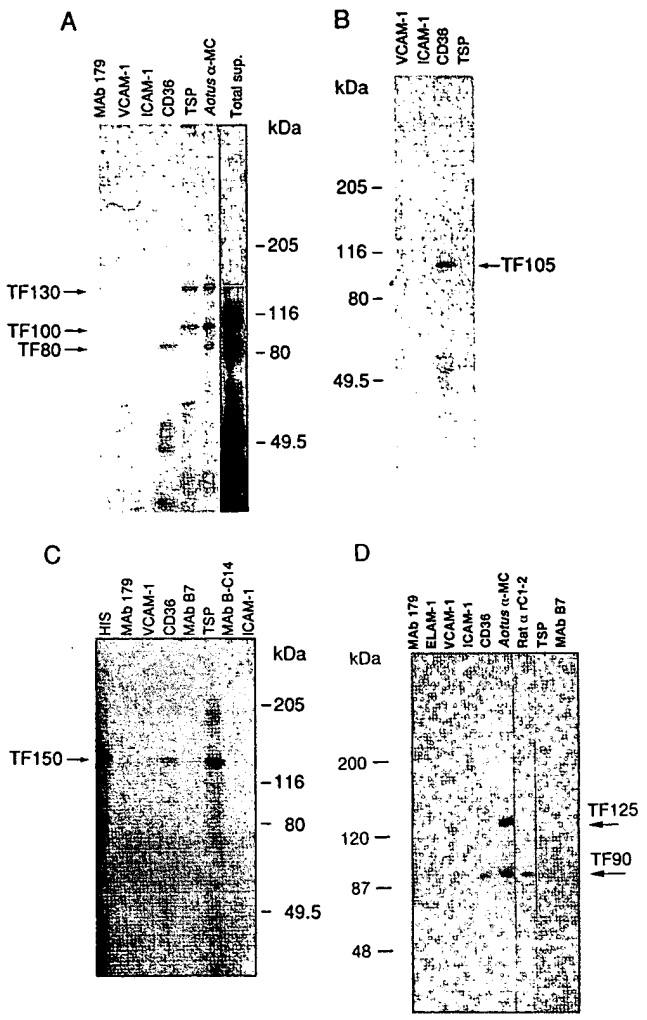

FIG. 3. Autoradiograph of tryptic fragments immunoprecipitated or affinity purified with immobilized host cell receptors that were derived from tryptic supernatants after mild trypsinization (L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, 10 μg/ml 5 min at room temperature) of surface-iodinated intact PRBCs. (*A*) Strain MC K^+C^+. (*B*) Clone FCR_3-C5. (*C*) Clone ItG2-ICAM. (*D*) Strain MC K^+C^+. Total sup., total supernatant of the tryptic fragments. *Aotus* α-MC, *Aotus* serum 9025 in *A* and *Aotus* serum 862 in *D*. Rat α rC1-2, serum of rat #1 reactive with the MC-PfEMP1–glutathione *S*-transferase fusion protein rC1-2.

with the smaller, trypsin-resistant PfEMP1 band (Figs. 2 and 4), which was not recognized by rabbit 05-75 serum (D.I.B., unpublished results). Preclearing the SDS extract with CD36 diminished the binding of PfEMP1 to CD36 by >95%, decreased the binding of PfEMP1 to TSP by 79%, and reduced the immunoprecipitation of PfEMP1 by anti-PfEMP1 sera by >80%. Preadsorption of the SDS extract with TSP reduced the binding of PfEMP1 to TSP and CD36 by 77% and 40%, respectively. The immunoprecipitation of PfEMP1 by *Aotus* anti-MC serum and rabbit 05-75 serum was decreased by 70% and 40%, respectively. Taken together, these results suggest that PfEMP1 possesses binding sites for both CD36 and TSP.

DISCUSSION

PfEMP1 plays a central role in *P. falciparum* biology and pathology. Because much of the pathology of *P. falciparum* is related to the ability of PRBCs to sequester in the deep vasculature, there has been great interest in the PRBC receptor as a vaccine target and for development of novel antiadherence therapeutic agents (3, 4). When PfEMP1 was first

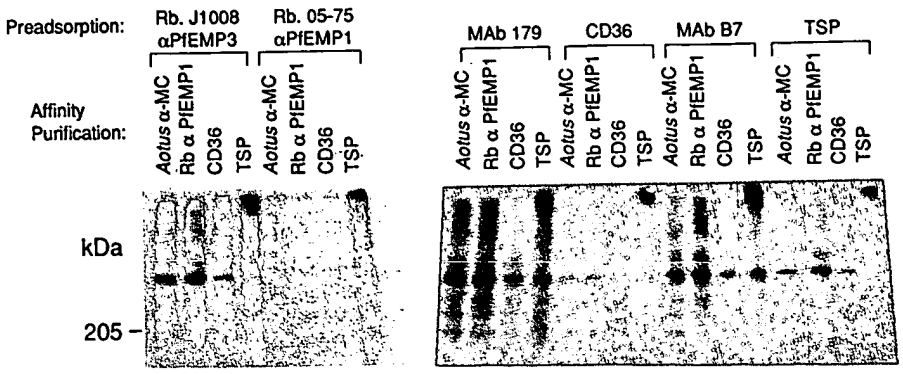

FIG. 4. Autoradiograph of PfEMP1 from total SDS extracts of MC K^+C^+ PRBCs immunoprecipitated or affinity purified after preadsorption. Four microliters of SDS extract was preadsorbed with protein G-Sepharose beads coated with rabbit anti-PfEMP3 serum (Rb. J1008 αPfEMP3), rabbit anti-PfEMP1/PfEMP3 serum (Rb. 05-75 αPfEMP1), MAb 179 alone (MAb 179), CD36 immobilized via MAb 179 (CD36); MAb TSP-B7 alone (MAb B7), or TSP immobilized via MAb B7 (TSP). After preadsorption, the extract was affinity purified with *Aotus* anti-MC K^+ sera 779 (*Aotus* α-MC), rabbit 05-75 anti-PfEMP1 (and PfEMP3) serum (Rb. αPfEMP1), immobilized CD36 (CD36), or TSP. Intensities of the relevant PfEMP1 bands are given in Table 1.

identified in 1984 (17), it had many features that correlated with the adherence properties of mature intact PRBCs (3, 14, 17). More recently, rat sera raised against a recombinant protein derived from the sequence of a MC PfEMP1 gene were shown to block adherence of MC strain PRBCs to CD36 (8). To identify the adherence receptor expressed on the surface of PRBCs, we investigated the binding of ^{125}I-labeled molecules from SDS and TX100 extracts of surface-iodinated PRBCs to immobilized host cell receptors. Protein bands that were affinity purified specifically by the endothelial cell receptors CD36, TSP, and ICAM-1 had molecular features identical to those of PfEMP1. These PRBC receptor proteins were surface iodinated, extractable only with SDS, variable in size between different strains, highly sensitive to trypsin, and had the same SDS/PAGE mobility as PfEMP1. Earlier studies showed that binding of PRBCs to CD36 is sensitive to pH and that calcium is required for adherence of PRBCs to TSP (22). All these properties of interaction of PRBCs with CD36 and TSP are mirrored in the results of the procedure applied here for the direct interaction of detergent-extracted PfEMP1 with TSP (Fig. 1*A*) and CD36 (D.I.B., unpublished data). The direct correlation between the interaction of PfEMP1 with each of the host receptors, and the adherence phenotype of the infected erythrocyte from which it was extracted, emphasize that these interactions are relevant to PRBC adherence. Thus, PfEMP1 is a PRBC adherence receptor for CD36, TSP, and ICAM-1.

Based on the parallel sensitivity of PfEMP1 and PRBC cytoadherence to trypsin (17), it was suggested that the same molecule might mediate PRBC adherence to the different host cell receptors. We found that PfEMP1 can bind to CD36, TSP, and ICAM-1. Preadsorption of the PRBC adherence receptor on either CD36 or TSP resulted in lower binding to the second counter-receptor (78% and 47%, respectively). Furthermore, a single tryptic fragment (Tf150) derived from the ItG-ICAM clone bound to both CD36 and TSP. Hence, the binding sites for CD36 and TSP are physically linked on the same PfEMP1 molecule. We conclude that a single PfEMP1 molecule can bear binding sites for CD36 and TSP, and possibly for ICAM-1 as well. In MC parasites, the CD36 and TSP binding domains were shown to reside on different (tryptic) fragments of PfEMP1 and therefore represent separate entities. This concept is supported by the observation that sera against the rC1-2 recombinant protein of MC strain PfEMP1 immunoprecipitate the CD36-binding fragment and block PRBC adherence to CD36 but have no effect on adherence to TSP (8).

PRBCs of different strains and isolates display a variety of adherence phenotypes (5, 18, 19, 24). Spontaneous phenotypic switching of surface PfEMP1 is often associated with acquisition of a new adherence phenotype (15, 16) and expression of a new PfEMP1 gene (11). Inspection of the published sequences of PfEMP1 *var* genes (8, 12) revealed that known binding motifs for integrins, such as LDV, RGD, and others, are present in different regions of some, but not all, PfEMP1 proteins (8, 12). Thus, a large and diverse pool of PfEMP1 genes (12), a high rate of phenotypic switching (2% per generation) (15), selection for expression of particular *var* genes, and adherence phenotypes can explain the plasticity of PRBC adherence *in vitro* and *in vivo* (5, 15, 18, 19, 25). This is consistent with our observation that the CD36- and TSP-binding domains are localized on tryptic fragments of variable sizes not only with different strains but also with different PfEMP1 forms from the same strain.

The domains of PfEMP1 involved with binding to CD36 and TSP are immunoprecipitated in a strain or isolate-specific

Table 1. Quantitative analysis of the binding of MC K^+ PfEMP1 to CD36 and TSP after preadsorption

Affinity purification	Anti-PfEMP1 serum			CD36			TSP		
	Control Rb α-PfEMP3*	Adsorption Rb α-PfEMP1†	Binding,‡ % of control	Control MAb 179	Adsorption CD36	Binding, % of control	Control MAb B7	Adsorption TSP	Binding, % of control
Aotus α-MC sera	12.90	1.03	7.98	19.75	3.88	19.65	9.46	2.86	30.23
Rb α-MC sera	17.66	0.29	1.64	23.50	3.03	12.90	14.83	9.40	63.39
CD36	7.15	0.10	1.40	10.89	0.43	3.95	4.20	2.38	53.67
TSP	6.07	1.66	27.35	10.79	2.32	21.50	6.50	1.50	23.08

The densitometry of autoradiographs from Fig. 4 exposed for 14 days is given as OD × mm², with SD < 10%. α, anti; Rb, rabbit. MAb 179 and MAb B7 were used to immobilize CD36 and TSP, respectively.

*Rabbit J1008 anti-PfEMP3 serum.

†Rabbit 05-75 anti-PfEMP3 and anti-PfEMP1 serum.

‡Binding remaining after preadsorption expressed as a percentage of binding relative to the appropriate control (nonrelevant or immobilizing antibody).

manner. Blockade of adherence by anti-*P. falciparum* sera or anti-PfEMP1 sera is strain-specific as well (8, 14, 26). Primates and humans infected with *P. falciparum* both develop adherence blocking antibodies (14, 17, 26). Interestingly, several *Aotus* and human sera tested did not immunoprecipitate the smallest CD36 binding fragments (≈20 kDa) of PfEMP1 (Fig. 3 and D.I.B., unpublished observations). Hence, the actual CD36-binding site as expressed on the surface of PRBCs appears not to elicit high-titer antibodies in *Aotus* monkeys or humans.

In conclusion, we have presented direct evidence for binding of PfEMP1 to the endothelial cell receptors CD36, TSP, and ICAM-1. PfEMP1 is therefore a malarial receptor that mediates adherence of PRBCs to microvascular endothelium. The binding sites on PfEMP1 and the cognate host cell receptors could conceivably be used for developing agents for reversal of PRBC adherence and consequent occlusion of microvessels, a potential treatment for acute cerebral malaria.

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